Epizoötic Vesicular Stomatitis in Colorado, 1982: Isolation of Virus from Insects Collected Along the Northern Colorado Rocky Mountain Front Range

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ABSTRACT Field studies were conducted during an epizoötic of vesicular stomatitis virus (VSV) in Colorado to further assess the possible role of insects in the transmission of VSV. Insects associated with domestic livestock were collected at 11 premises along the Front Range of the Rocky Mountains in Colorado during the 1982 epizoötic of vesicular stomatitis. Insects were pooled by date, location, species, and sex and were processed for virus isolation in three cell culture systems. Thirty-four isolates of vesicular stomatitis virus, New Jersey serotype, were obtained from 51,036 insects. Of these, 27 isolates were from *Musca domestica* (126 pools/5,285 specimens), 5 from other nonhematophagous Diptera (56 pools/936 specimens), and 2 from unengorged black flies (Simuliidae) (55 pools/1,221 specimens). Results suggest that nonblood-feeding insects, such as houseflies, play a role in VSV transmission and that black flies also serve as vectors.

KEY WORDS Insecta, VSV, VSV from nonhematophagous insects, VSV from Simuliidae

VESICULAR STOMATITIS (VS) is a disease of cattle, horses, swine, and various wild vertebrates; it is caused by several viruses of the family Rhabdoviridae, genus *Vesiculovirus*, found in North and South America and Asia (Yuill 1981). VS is enzoötic in the tropical and subtropical regions of the Americas, but it can spread northward into the United States and Canada during the summer months, appearing first in states along the Gulf of Mexico in April or May and later as far north as Manitoba, Canada.

The natural history of VS virus in North America, including endemic maintenance and epizoötic transmission, remains uncertain despite many years of study. Outbreaks often have appeared more or less simultaneously within a broad geographical area. The seasonal occurrence of disease and the spotty geographic distribution of cases suggest insect transmission. VS Indiana (VSI) virus has been isolated frequently from sand flies (Diptera: Psychodidae) caught in the wild in tropical America (Yuill 1981) and from mosquitoes (Diptera: Culicidae) collected in New Mexico (Sudia et al. 1967). Replication of the virus and transmission by bite have been demonstrated in sand flies (Tesh et al. 1971), and high rates of vertical (transovarial) transmission also have been found (Tesh et al. 1972).

The New Jersey serotype (VSNJ) has been isolated from black flies (Diptera: Simuliidae) in Colombia (Theiler & Downs 1973) and from mosquitoes collected in Ecuador (Calisher et al. 1983) and Guatemala (C.H.C., unpublished data). During a 1966 epizoötic outbreak in Colorado, VSNJ virus was isolated from *Hippelates* eye gnats (Diptera: Chloropidae) (Jenny 1967). Ferris et al. (1955) mechanically transmitted VSNJ virus with *Stomoxys calcitrans* (L.), several species of Tabanidae, and four species of Culicidae.

The 1982 VSNJ outbreak started in Arizona in May 1982 and eventually affected livestock in 14 states. The major features of this outbreak have been described previously (Webb et al. 1987, Monath et al. 1987). We report here virus isolations from insects collected along the Front Range, the eastern side of the Rocky Mountains between Denver and Fort Collins, Colo., during the 1982 outbreak. The intent of this investigation was to determine which insect species, if any, were serving as virus vectors during the epizoötic.

Materials and Methods

Field studies were conducted at 11 locations north of Denver in Adams, Boulder, and Larimer counties, on or near premises where domestic livestock were then ill with VS (Fig. 1). The study included areas where primarily dairy cattle were affected (Sites 1 and 3), where beef cattle and horses were affected (Sites 4, 5, and 7), and where only horses were involved (Sites 2, 6, 8–11). Elevations of the study sites ranged from 4,900 ft to 5,200 ft.

Entomological investigations were aimed at collecting a wide variety of possible insect vectors. These included not only Diptera associated with

livestock but also plant-feeding insects belonging primarily to the order Homoptera and other insect orders. Insects were collected with Centers for Disease Control (CDC) miniature light traps (Hausherr's Machine Works, Toms River, N.J.) with or without CO_2. CDC light traps were hung in trees or suspended from 1.8-m fence posts with a short arm welded onto the top. Two traps were hung from the arm of each post, one at approximately 1.5 m above the ground and one as low as possible, with the collection bag just clearing the ground surface. Sweep net collections were made from vegetation and from various structures associated with livestock pens and maintenance facilities including feed bunks and the insides of barns. Insects also were aspirated directly from affected livestock. A limited number of collections were made with a Malaise trap (BioQuip Products, Santa Monica, Calif.).

Specimens from each trap or collection method and location were put in individual vials and transported on dry ice to the Division of Vector-Borne Viral Diseases laboratory, CDC, Fort Collins, Colorado. In the laboratory, insects were identified and pooled on refrigerated chill tables to prevent loss of virus. Where possible, insects were identified by species; however, some specimens were identified only to genus or family. Representative black flies were sent to the National Museum of Natural History (Washington, D.C.).

Pooled insects were triturated in 2.0 ml of diluent composed of 1% bovine albumin in pH 7.6 Tris-buffered saline containing antibiotics. Suspensions were clarified by low-speed centrifugation, and the supernatant fluids were either assayed immediately or were stored at −65°C for later testing. Two-tenths milliliter of the supernatant fluids from each arthropod pool was spread onto monolayer cultures of primary Pekin duck (DE) and a continuous line of African green monkey kidney (Vero) cells grown at 37°C in 25-cm^2 flasks and into tube cultures of *Aedes albopictus* (C6/36) cells. After adsorption of inocula, DE and Vero cultures were overlaid with a nutrient agar (Hayes et al. 1972). Medium was poured from tube cultures of C6/36 cells, inocula were added and adsorbed for approximately 1 h, and liquid maintenance medium was added. The C6/36 cultures were incubated at 22°C for 3 d, frozen and thawed once, and the suspension then was inoculated onto monolayer cultures of DE and Vero for virus detection by plaque assay. Cytopathogenesis in the C6/36 cultures was not noted by day 3. Recovered virus strains were identified by complement-fixation (Casey 1965), neutralization tests (Lindsey et al. 1976), or both.

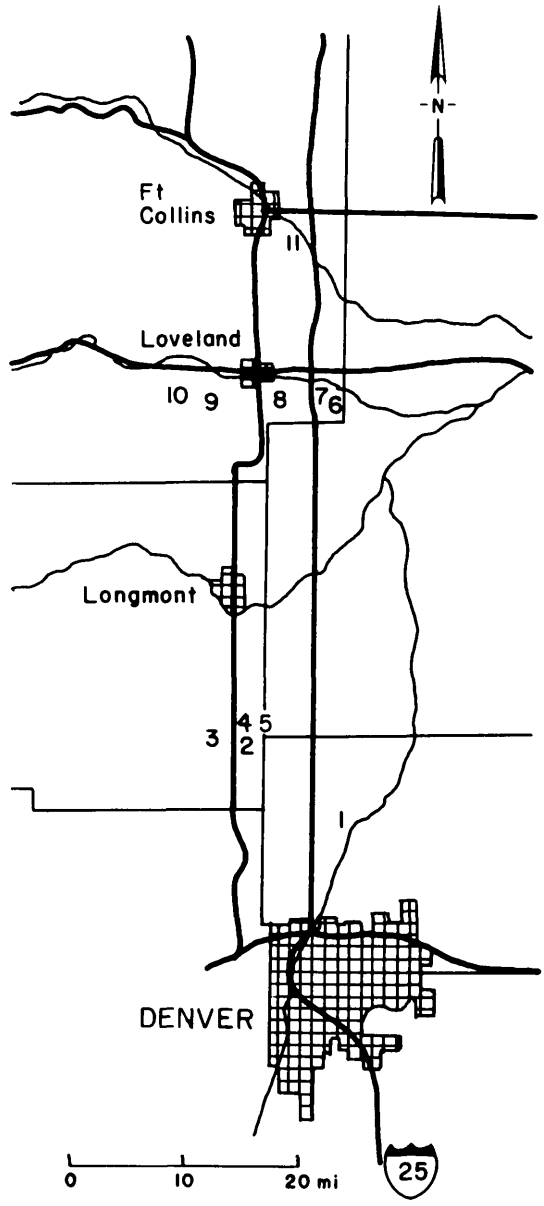

Fig. 1. Rocky Mountain Front Range study sites (1–11) used during field investigations of epizoötic VS in Colorado, 1982.

Results

More than 51,000 insects were assayed for virus (Table 1). VSNJ virus was recovered from 34 pools (Table 2). Had testing been conducted using only DE and Vero cell cultures, 14 virus strains would have been isolated. An additional 20 virus strains were obtained by using C6/36 cell cultures to amplify virus before detection in DE or Vero cell culture. One strain was isolated only in DE cells, and none was isolated only in Vero cell cultures.

Virus-positive insect pools were collected at two sites by using several collection methods (Table 3). Most of the virus strains were obtained from houseflies, which were collected primarily with sweep nets near livestock feed bunks and from walls inside barns or other outbuildings on premises with affected animals. Both virus isolations from black flies

Table 1. Insects collected and tested during studies of VS along the Rocky Mountain Front Range, Colorado, 1982

Order Family	No. tested	
	Specimens	Pools
Diptera		
Psychodidae	1,994	7
Ceratopogonidae (2 genera, 7 species)	14,278	112
Simuliidae (1 genus, 2 species)	1,221	55
Culicidae (3 genera, 12 species)	18,887	463
Chloropidae (1 genus)	61	10
Anthomyiidae	875	46
Muscidae (4 genera, 3 species)	5,426	171
Other Diptera (13 families)	880	70
Hemiptera		
Miridae	299	35
Lygaeidae	114	11
Nabidae	69	12
Other Hemiptera (3 families)	40	10
Homoptera		
Cicadellidae	4,550	75
Aphididae	2,089	30
Other Homoptera (4 families)	196	18
Hymenoptera		
(2 families)	20	7
Coleoptera		
(1 family)	33	11
Orthoptera		
(1 family)	4	1
Total	51,036	1,144

were from specimens containing no visible blood, which were collected in CDC light traps. One of the positive pools lysed the cell culture monolayer on primary isolation, providing evidence that the pool contained $>10^{3.3}$ plaque-forming units. Black flies in the virus-positive pools were not identified to species; however, voucher specimens from the collections were identified as predominantly *Simulium vittatum* Zetterstedt and *S. bivittatum* Malloch. The chloropid flies were mostly *Hippelates* spp.

In addition to the VSNJ virus isolations, western equine encephalomyelitis (WEE), Turlock (TUR), California group (CAL), and Hart Park (HP) viruses were recovered from mosquitoes (Table 2). All three viruses WEE, HP, and TUR, were recovered from *Culex tarsalis* Coquillett. TUR and HP viral strains were recovered from *Cx. pipiens pipiens* L., and HP virus and an untyped CAL group virus were isolated from a pool of *Ae. vexans* (Meigen).

Discussion

Epidemiologic observations of epizoötics over a long period have led to the hypothesis that insects may be important in transmitting VS viruses (Yuill 1981). We have shown that several species of biting and nonbiting flies can acquire VSNJ virus in nature. This lends support to the hypothesis that insect vectors are involved in animal-to-animal transmission. Two possible mechanisms of transmission are suggested by the data: mechanical transmission by either biting or nonbiting flies and biological transmission by biting flies.

In 1897, Theiler (Ferris et al. 1955) demonstrated that VS virus was transmitted from horse to horse through minute abrasions of the mucous membranes. Because they feed on roughage, cattle and horses are likely to have small abrasions and lesions on and around their mouths most of the time. Fluids removed from vesicular lesions of affected animals contain very high concentrations of virus (Hanson & Brandly 1957). Most probably, positive flies obtained in this study acquired virus while feeding from lesions on affected animals. The number of virus isolations from houseflies and the frequency with which we observed them feeding around the eyes and the mouth lesions of affected animals suggest that they are important in the spread of virus, even if they are not particularly efficient transmitters.

Whether or not nonbiting flies, such as *Musca*

Table 2. Insects from which virus strains were isolated during studies of VS along the Rocky Mountain Front Range, Colorado, 1982

Group	No. tested		Virus isolations[a]				
	Specimens	Pools	VSNJ	WEE	HP	TUR	CAL
Culicidae							
Aedes vexans	5,412	90			1 (0.2)		1 (0.2)
Culex pipiens	1,031	33			2 (1.9)	1 (1.0)	
Cx. tarsalis	2,957	68		4 (1.4)[b]	8 (2.7)	5 (1.7)	
Simuliidae	1,221	55	2 (1.6)				
Chloropidae	61	10	1 (16.4)				
Anthomyiidae	875	46	4 (4.6)				
Muscidae							
Musca domestica	5,285	126	27 (5.1)				
Total	16,842	428	34	4	11	6	1

[a] VSNJ, vesicular stomatitis (New Jersey); WEE, Western equine encephalomyelitis; HP, Hart Park; TUR, Turlock; CAL, California group.

[b] Minimal infection rate/1,000 insects.

Table 3. Insects from which VSNJ virus was isolated along the Rocky Mountain Front Range, Colorado, 1982

Site	Date	Species	Virus isolation by collection method		
			Sweep net	Light trap	Landing biting
9	9 Sept. 1982	*Musca domestica*	2		1
		Anthomyiidae	2		2
		Simuliidae		2	
		Chloropidae			1
10	16 Sept. 1982	*Musca domestica*	24		

and *Hippelates*, that feed around lesions could transmit VS virus by feeding on abrasions or on mucous membranes around the eyes, remains to be proven. VSNJ virus has been recovered from *Hippelates* flies (Jenny 1967), which have been implicated as mechanical vectors of several other human and animal pathogens (James & Harwood 1969). Additionally, clinical disease in horses and cows can be reproduced only by injection of virus into the tongue or mucous membranes, whereas subcutaneous, intramuscular, intravenous, or other parenteral routes of inoculation result in immunization without disease (Tesh & Johnson 1975). These observations indicate that mechanical transmission by nonbiting flies from mucous membrane to mucous membrane may be required to produce clinical disease.

Epidemiologic observations on one of the horse ranches (Site 10) suggest possible insect involvement in animal-to-animal transmission. Horses, kept in separate stalls with no common feed or water source, had onset of clinical disease over a period of 14 d (Webb et al. 1987). Direct contact almost certainly could be eliminated as a mode of transmission in these animals, whereas houseflies, which were abundant on the walls of the barns and stalls despite the frequent use of insecticide, may have disseminated virus.

Delivery and pickup trucks used at dairies contained numerous resting muscoid flies inside cabs and cargo spaces, providing a plausible mode for spreading infected insects from one premise to another. Because these vehicles usually visit several premises in the same day, infected insects could be spread over relatively long distances. This is consistent with the usually spotty distribution of premises with infected animals (Jonkers 1967, Yuill 1981).

While any housefly transmission is likely to be mechanical, blood-feeding insects may serve as biological vectors of VSNJ virus in North America, as phlebotomine sand flies do for VSI virus in Central America (Yuill 1981). Our finding of VSNJ virus in black flies supplements a previous report of VSNJ virus isolation from *Simulium* sp. in Colombia (Theiler & Downs 1973). The recovery of relatively high concentrations of VSNJ virus from one of the black fly pools on original isolation suggests that virus replication may have occurred within one or more flies. Alternatively, one or more flies might have recently fed on vesicular fluids containing large amounts of virus.

The isolation of VSNJ virus in black flies, which breed in riparian habitats, is interesting because of the observation that VS outbreaks tend to be associated with river valleys (Hanson 1952). This association also appeared to be true to some degree in Colorado, although epidemiologic studies to confirm it were not conducted. In contrast to previous studies (Sudia et al. 1967), we did not recover VS virus from any of the collected mosquitoes, although a number of other viruses were isolated from those mosquitoes.

Field and laboratory observations show that horses and cattle do not develop a high or consistent viremia during infection (Yuill 1981). Therefore, it seems unlikely that large amounts of this virus are available to hematophagous insects during blood feeding unless they coincidentally feed on exudates from vesicular lesions, which frequently contain large amounts of virus. Moreover, transmission by blood-feeding insects would be expected to produce immunizing, but not overt VS unless insect bites occurred around the nose and mouth.

Despite laboratory demonstration that a number of dipteran species can mechanically transmit VSNJ virus (Ferris et al. 1955) and the relatively large number of viruses isolated from insects during the 1982 Colorado epizoötic, the epizoötiology of VSNJ remains an enigma. Our observations lend further support to the hypothesis that insects are involved in the natural transmission cycle of VSNJ virus.

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